Clinical Validity: Prenatal Screening for Cystic Fibrosis

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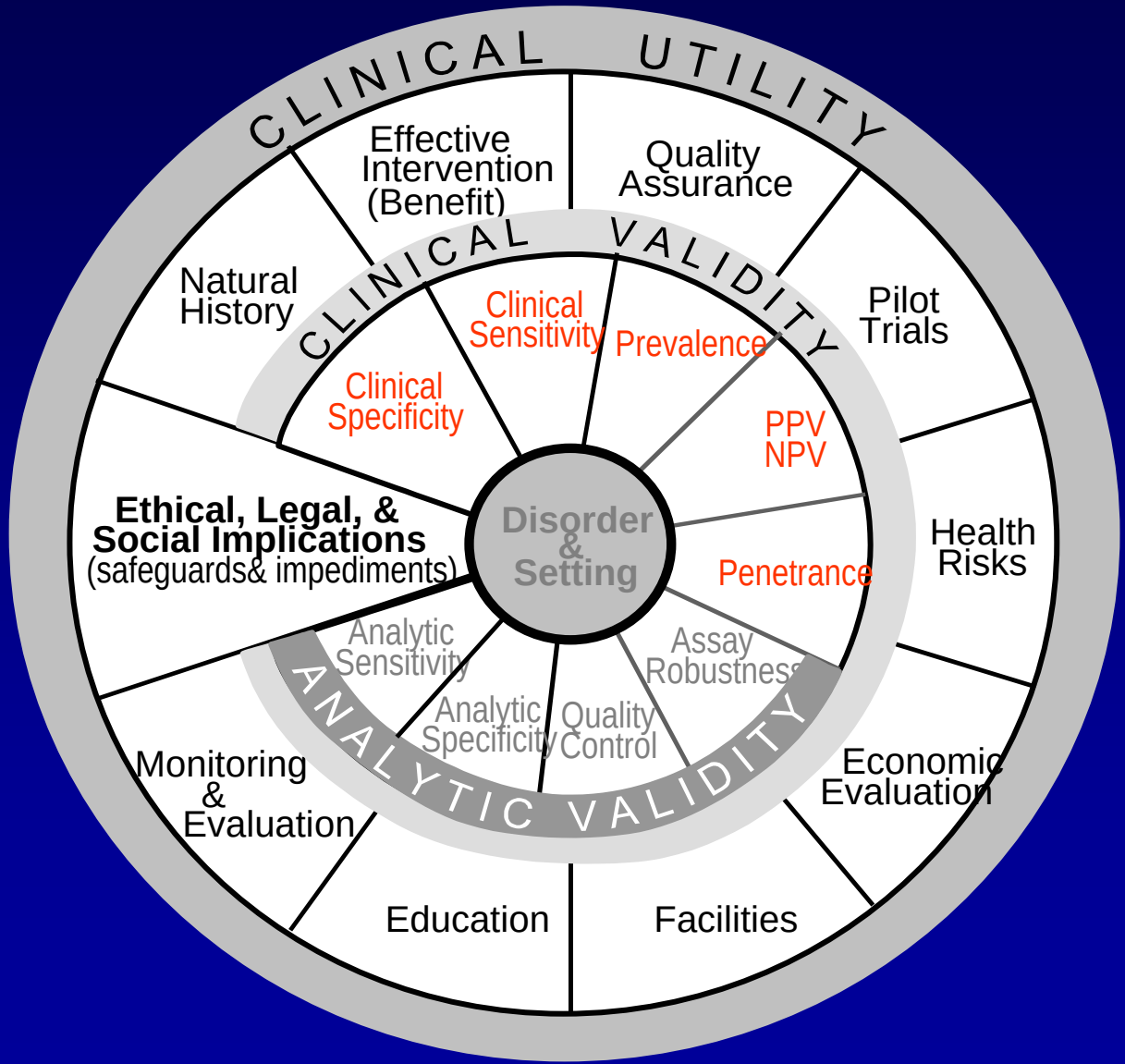
Clinical Validity

- Defines the ability of a test to detect or predict the phenotype or particular clinical outcome
- Elements build upon analysis of analytic validity for a specific disorder in a defined setting



Clinical Sensitivity & Specificity

Test Result	Disease Phenotype: Yes	Disease Phenotype: No
Pos	A	B
Neg	C	D

Sensitivity: Proportion of persons with the disease who have a positive test = A / (A+C)

Specificity: Proportion of persons who do not have the disease who have a negative test = D / (B+D)

Issues in evaluating Clinical Validity

- Study design
  - Case definitions may vary
  - Small numbers and potential biases
  - Populations may need to be stratified by variables such as gender, age, race/ethnicity
  - Testing protocols may not be comparable (e.g., numbers of mutations tested)
  - Comparability of case and control populations
- Need to evaluate the impact of genetic and environmental modifiers

Clinical Sensitivity of *CFTR* Testing: Prenatal Screening for Cystic Fibrosis

- Clinical sensitivity **is** dependent on the panel of *CFTR* mutations selected
  - This review will focus on the ACMG recommended panel of 25 mutations
- Clinical sensitivity **is** dependent of the race/ethnicity of the population screened
  - This review will provide estimates for self-reported race/ethnic groups (i.e., Hispanic and non-Hispanic Caucasians, Ashkenazi Jewish, African Americans and Asian Americans)
- Clinical sensitivity **is not** dependent on the screening model used
  - Sequential (two-step), couple (one-step) and concurrent

American College of Medical Genetics (ACMG) Recommended Panel of 25 *CFTR* Mutations

- **7 mutations often tested in 7-13 pilot trials**
  - delF508, G551D, G542X, 621+1G>T, W1282X, N1303K, and R553X
- **7 mutations sometimes tested (2 to 6 trials)**
  - delI507, 1717+1G>T, R117H, 3849+10kbC>T, R560T, A455E, R334W
- **11 mutations rarely or never tested (0 or 1)**
  - R347P, 711+1G>T, R1162X, I148T, 2789+5G>A, 3569delC, G85E, 2184delA, 1898+1G>A, 3120+1G>T, 1078delT

To Reliably Estimate Clinical Sensitivity Requires

- An unbiased set of DNA samples from individuals with clinically defined ‘classic’ cystic fibrosis
- A set test panel of *CFTR* mutations
- Two large datasets are available:
  - The Cystic Fibrosis Consortium (diagnostic laboratories)
  - The Cystic Fibrosis Foundation (clinics providing patient care)

An ACCE Review Involves a Critical Evaluation of the Literature

- The CF Consortium (Kazazian et al., 1994) reported mutation frequencies in North Americans (Caucasians). However, those numbers could not be used directly for two reasons:
  - This group includes reports that focus mainly on Hispanic Caucasians, Ashkenazi Jewish or African Americans
  - There was a computational error that underestimated mutation frequencies, especially for those less common

Cystic Fibrosis Consortium Reanalysis

Mutation	Mutation Frequency (%) Original	Revised*
delF508	66.1	68.94
G542X	2.2	2.17
G551D	2.0	2.06
621+1G>T	1.5	1.91
?? Others	7.4	8.20
A455E	0.25	0.54
711+1G>T	0.20	0.77
R347P	0.25	0.46
All	**79.9**	**85.05**

***After removing 15 reports and using a new denominator**

Mutation Frequencies in Non-Hispanic Caucasians

Mutation	CF Consort	CF Found	Average	Cumulative
delF508	68.94	75.90	72.42	72.42
G542X	2.17	2.39	2.28	74.70
G551D	2.05	2.44	2.25	76.95
621+1G>T	1.92	1.22	1.57	78.52
W1282X	1.42	1.57	1.50	80.02
20 others	8.55	8.12	8.33	88.35
Total	**85.05**	**91.64**	**88.35**	

CF Consortium based on between 2,197 and 9,792 chromosomes

CF Foundation based on 3,938 chromosomes

Which Set of Mutation Frequencies Should be Used?

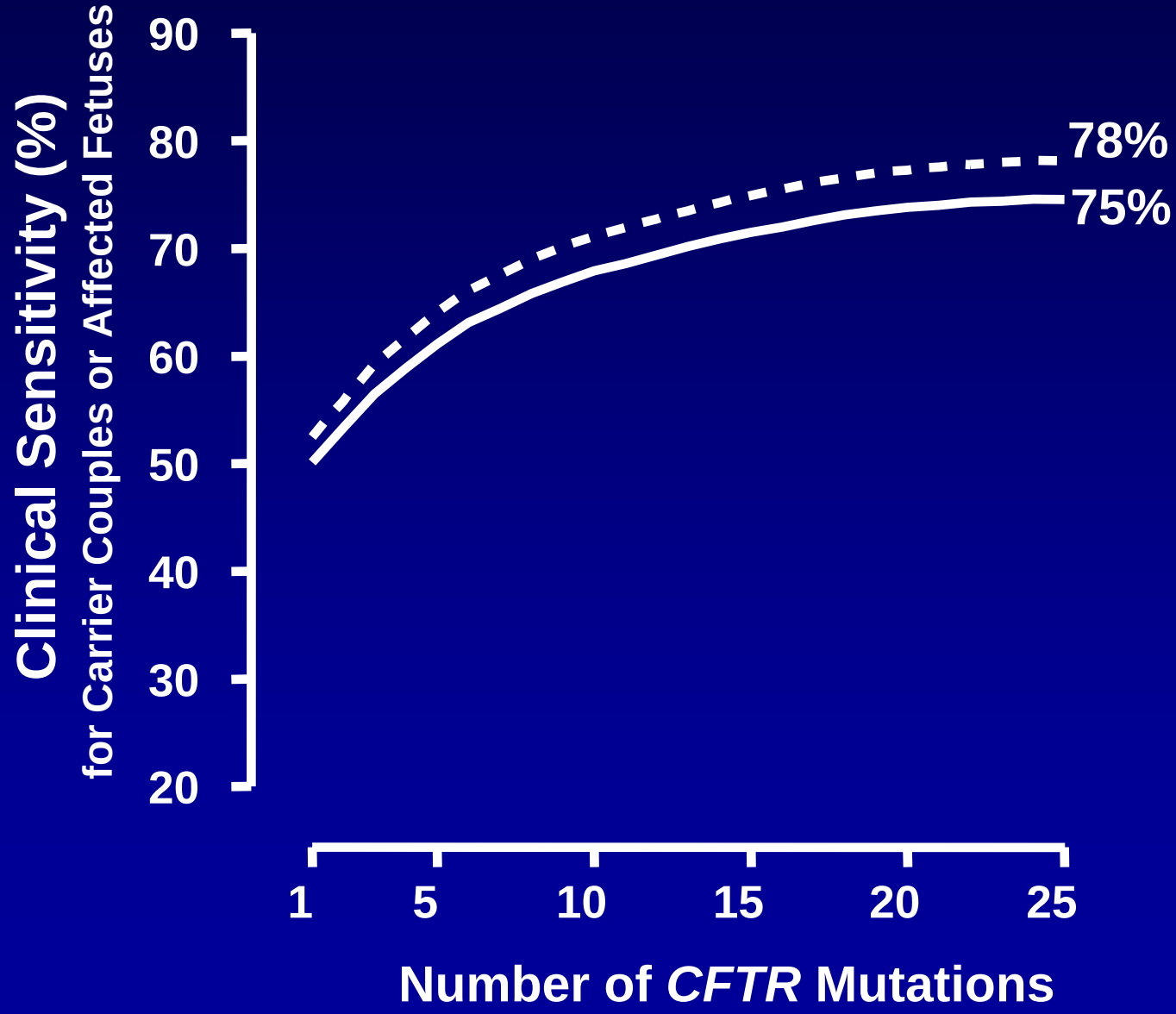
- The Cystic Fibrosis Consortium data may under-represent ‘common’ mutations such as delF508 because of the ‘reference’ nature of participating laboratories
- The Cystic Fibrosis Foundation data may over-estimate the more ‘severe’ mutations such as delF508, because they are more likely to be enrolled for services
- The average of the two may be reasonable to use

Relationship Between Carriers Identified & Detection of Carrier Couples (or Affected Fetuses)

Proportion Identified (%)		
Carrier Individuals	**Carrier Couples**	**Increase***
20	4	
30	9	5
40	16	7
50	25	9
60	36	11
70	49	13
80	64	15
90	81	17
95	90	9

* Increase in detection for 10% increase carriers identified

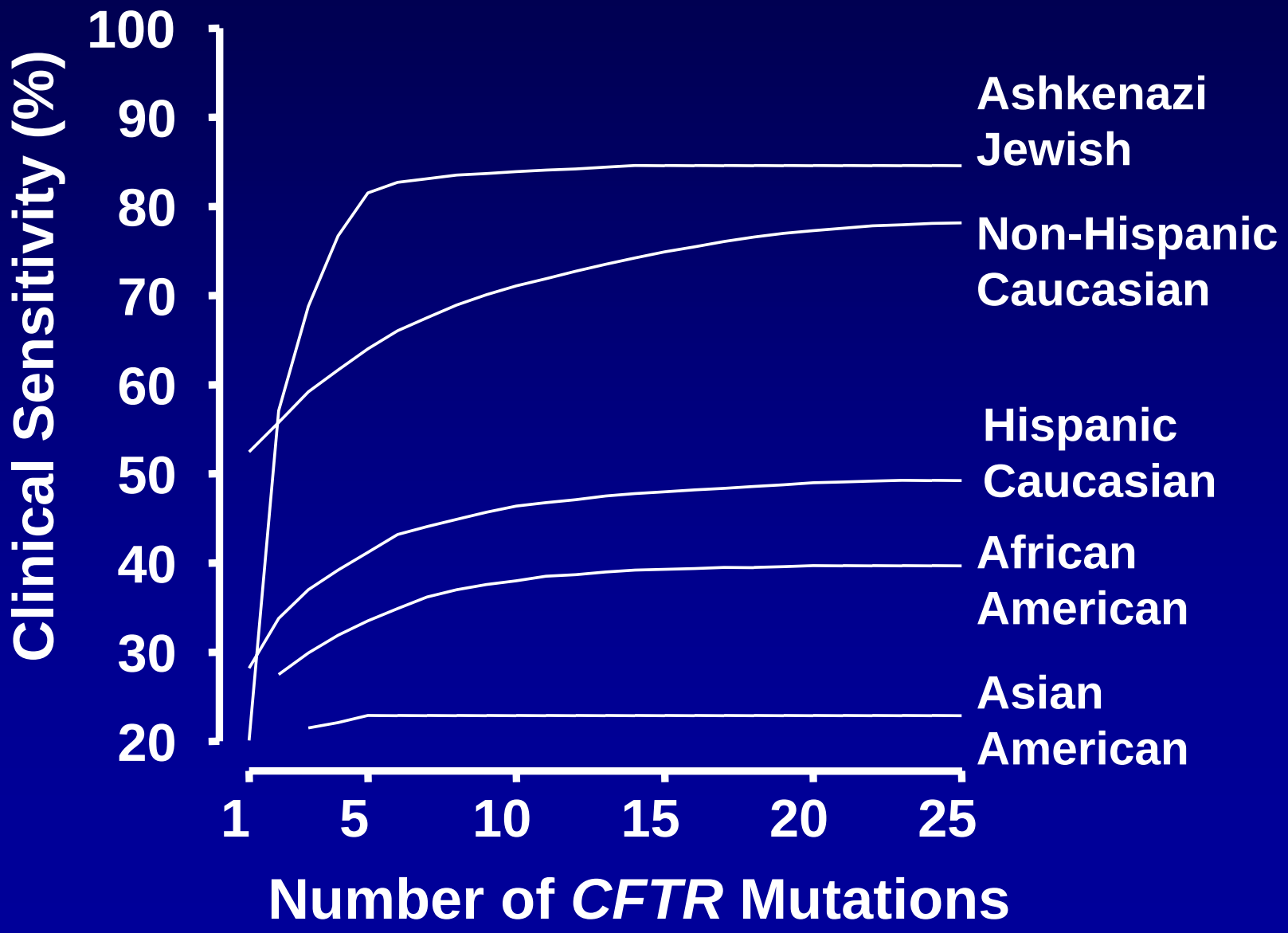
Carrier Couples Detected (Clinical Sensitivity) Among Non-Hispanic Caucasians



Clinical Sensitivity in Other Racial/Ethnic Groups

- Similar analyses are performed for individuals reported to be
  - Hispanic Caucasians
  - Ashkenazi Jewish
  - African American
  - Asian American

Clinical Sensitivity of Prenatal Cystic Fibrosis Screening by Race/Ethnicity



Clinical Specificity of Prenatal Screening for Cystic Fibrosis

- Analytic false positive results
  - Pre analytic
    - Sample mix-up prior to laboratory receipt
    - Degraded or mishandled sample
    - Non-paternity
  - Analytic
    - Sample mix-up after laboratory receipt
    - Benign polymorphism mistaken for a mutation
  - Post analytic
    - Data entry error
    - Incorrect laboratory interpretation
    - Report mix-up at the laboratory or provider site

Clinical Specificity of Prenatal Screening for Cystic Fibrosis

- Clinical false positive results
  - Incomplete penetrance
    - Can be minimized, but not eliminated, by reflexive testing associated with R117H and I148T (D1152H is not on the panel but is likely to be of low penetrance as well)
    - A small proportion (up to 5%?) of individuals with two mutations will not have the ‘classic’ CF phenotype
  - Mutation is not disease-causing
    - Possible that I148T is a polymorphism that is tightly linked to a ‘real’ mutation

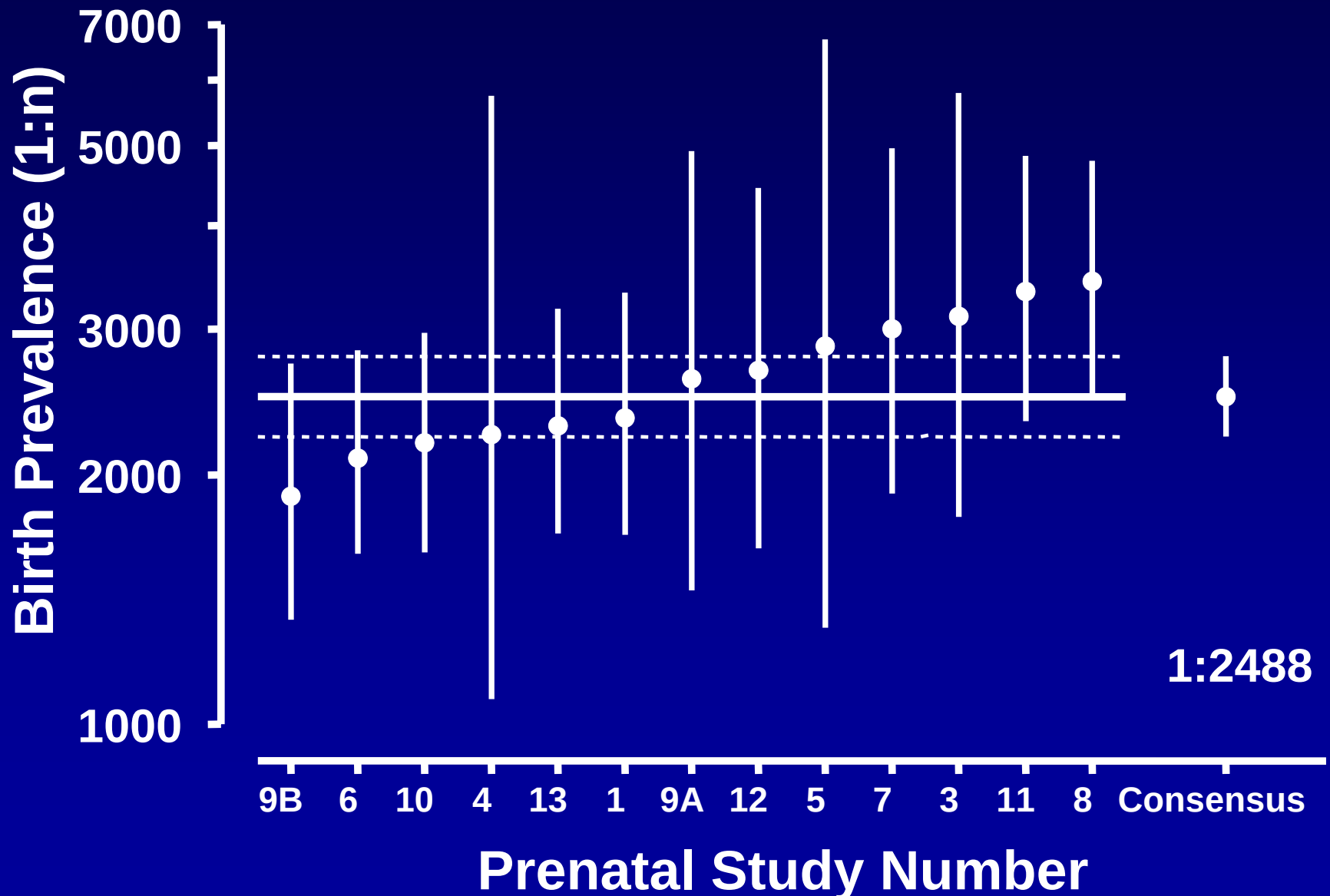
The Birth Prevalence of Cystic Fibrosis by Race/Ethnicity

- Sources of data include:
  - prenatal (pre) screening trials
  - newborn (new) screening trials
  - population (pop) registries
- Identified biases include:
  - number of mutations tested (pre, new)
  - race/ethnicity not accounted for (pre, new, pop)
  - prenatal screening and termination (new, pop)
  - length of follow-up (new, pop)
  - other study-specific biases (pre, new, pop)

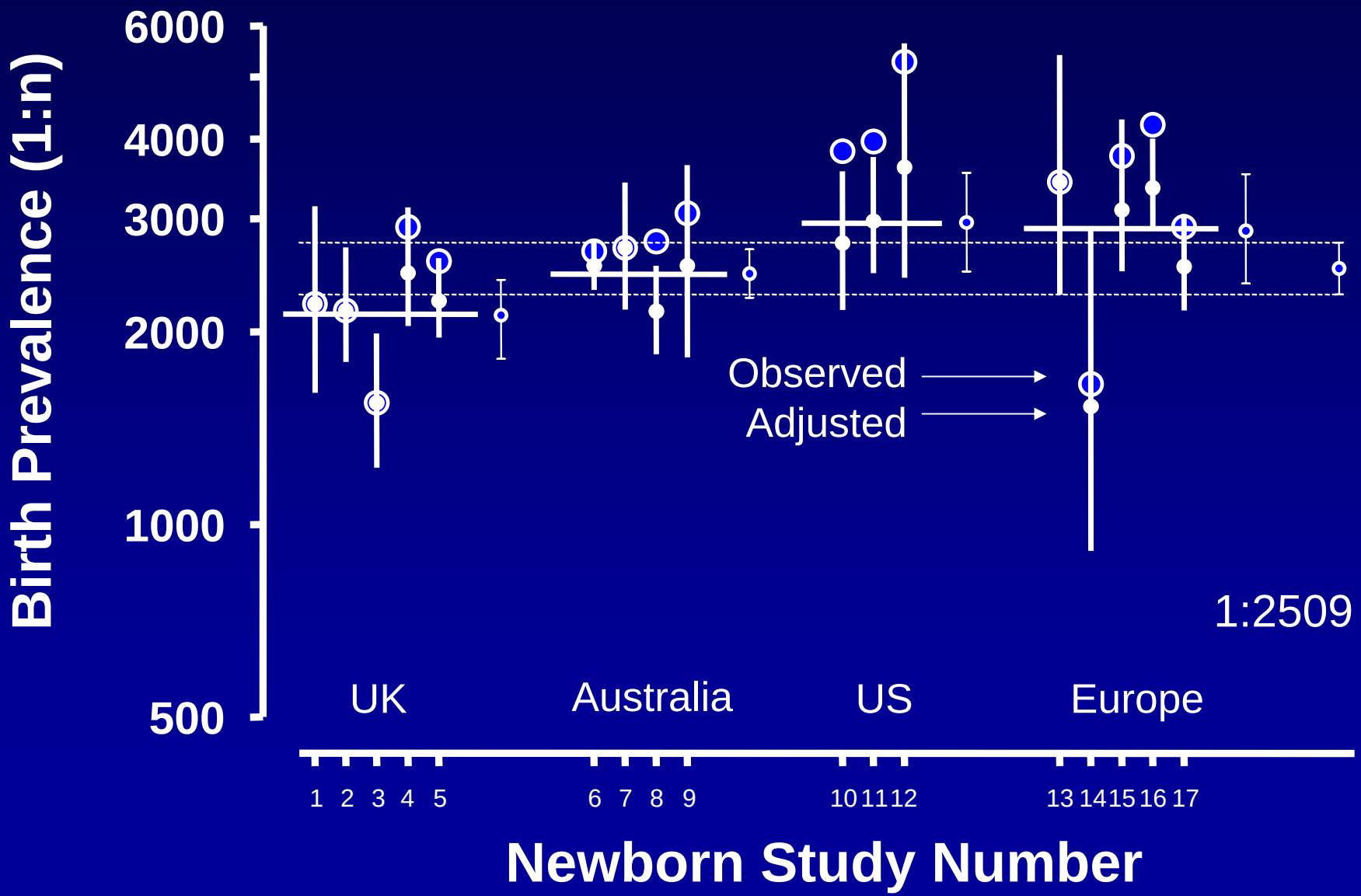
ACCE Methodology Attempts to Account for Biases Rather than Excluding Studies

- Number of mutations tested, and proportion of cases detected by race/ethnicity can be taken into account by using the race/ethnic specific mutation rates described earlier
- Several studies provide the relative rates of prenatal screening and termination that occurs in concert with newborn screening (Europe / Australia)
- The proportion of cases detected during reported follow-up can be adjusted using age at diagnosis information from the CF Foundation

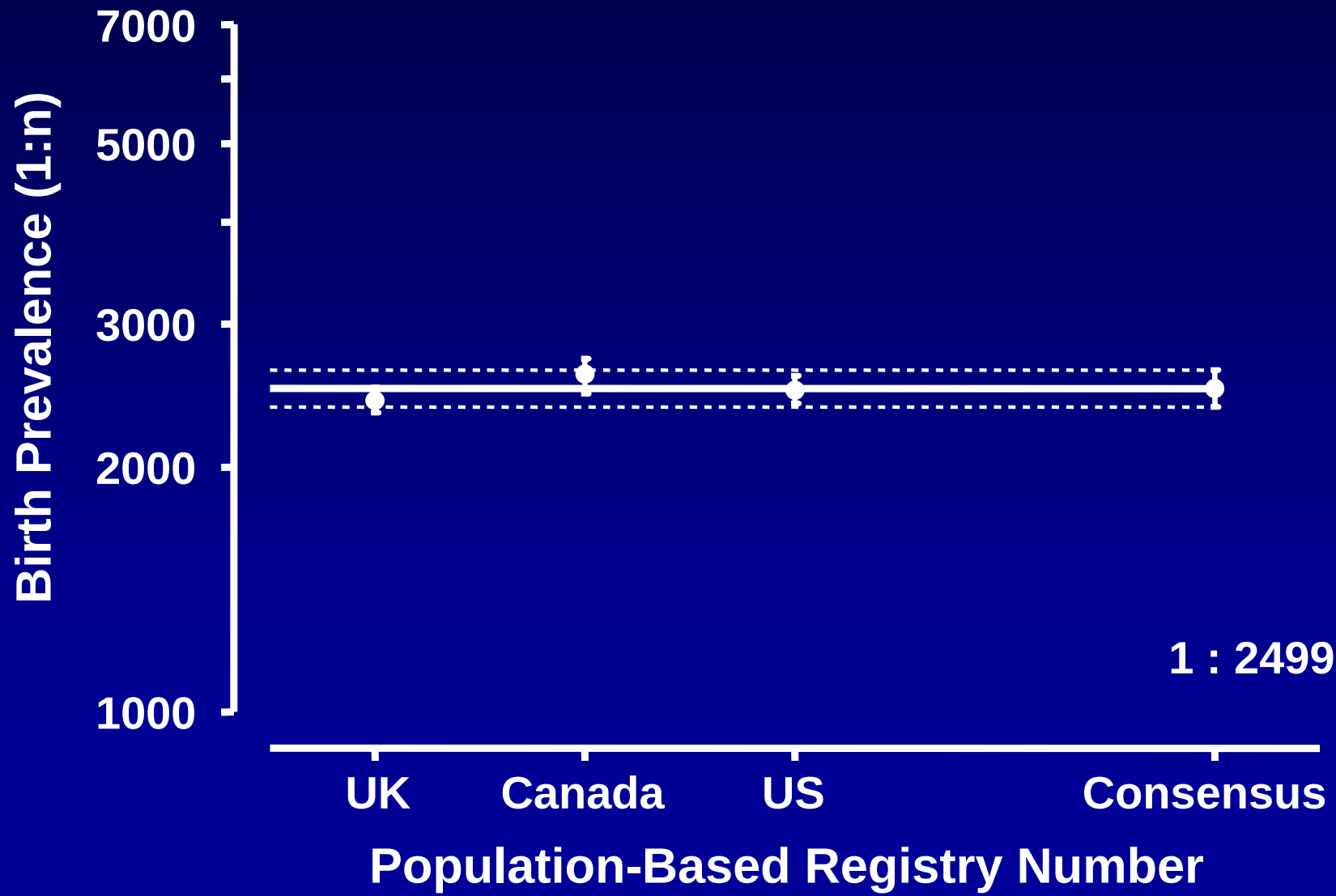
Adjusted Prevalence of CF in non-Hispanic Caucasians Using Prenatal Screening Trials



Prevalence of Cystic Fibrosis in non-Hispanic Caucasians Using Newborn Screening Trials



Prevalence of Cystic Fibrosis in non-Hispanic Caucasians Using Population Registries



Summary: Birth Prevalence of Cystic Fibrosis in Selected Racial/Ethnic Groups

Racial/Ethnic Group	Studies	Prevalence (1:n)
Ashkenazi Jewish	4	2,271
Non-Hispanic Caucasians	33	2,500
Hispanic Caucasians	3	13,500
African Americans	3	15,100
Asian Americans	1	31,000

Genotype/Phenotype Relationships

- ‘Classic’ cystic fibrosis phenotype occurs about 98% of the time when two mutations in the recommended mutation panel are identified.
- Most mutations associated with pulmonary disease, but cannot accurately predict timing of onset & progression.
- Pancreatic insufficiency in most cystic fibrosis patients, but 5 to15% have pancreatic function. Certain less common mutations are associated with pancreatic sufficiency.
- Screening is expected to generate more information.

Acknowledgements

- ACCE Review: Population-based prenatal screening for cystic fibrosis via carrier testing (available on the CDC website)
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